CLAIMS

What is claimed is:

1. An affinity fluorescent protein comprising a modified flourescent protein molecule which comprises a mutated flourescent protein molecule and a heterologous amino acid sequence, thereby introducing a ligand-activated protein binding site, wherein the modified flourescent protein molecule displays an altered spectral property when the binding site is engaged with ligand relative to the spectral property displayed when the binding site is not engaged by ligand.

2. An affinity fluorescent protein comprising a modified GFP molecule which comprises a mutated GFP molecule and a heterologous amino acid sequence, thereby introducing a ligand-activated protein binding site, wherein the modified GFP molecule displays an altered spectral property when the binding site is engaged with ligand relative to the spectral property displayed when the binding site is not engaged by ligand.

3. The affinity fluorescent protein of Claim 2 wherein the mutated GFP comprises a substitution of Ser147Pro.

4. The affinity fluorescent protein of ~~Claim~~ 2 wherein the altered spectral property is selected from the group consisting of: an altered absorption spectra, an altered excitation spectra, an altered emission spectra and any combination thereof.

5. The affinity fluorescent protein of Claim 4 wherein the modified GFP molecule comprises one or more protein binding sites introduced at a single site in tandem or introduced at distinct sites as separate heterologous sequences.

6. The affinity fluorescent protein of Claim 5 wherein the modified GFP molecule comprises protein binding sites introduced into a loop present on the surface of the GFP molecule and the presence of the heterologous amino acid sequences does not alter the spectral properties of the GFP.

7. The affinity fluorescent protein of Claim 6 wherein the modified GFP molecule comprises at least one heterologous amino acid sequence introduced at a location of the GFP molecule selected from the group consisting of: the N-terminus, between Gln157 and Lys158, between positions Glu172 and Asp173, the C-terminus and any combination thereof.

8. The affinity fluorescent protein of Claim 7 wherein the mutated GFP comprises a substitution of Ser147Pro.

9. The affinity fluorescent protein of Claim 8 wherein the affinity fluorescent protein comprises at least one protein binding site comprising a heterologous amino acid sequence introduced between Gln157 and Lys158 of the GFP molecule.

10. The affinity fluorescent protein of Claim 8 wherein the affinity fluorescent protein comprises at least one protein binding site comprising a heterologous amino acid sequence introduced between Glu172 and Asp173 of the GFP molecule.

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11. An affinity fluorescent protein expression cassette comprising a modified GFP nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein the modified GFP sequence comprises a recombinant peptide which comprises restriction endonuclease sites introduced at a location of the GFP molecule selected from the group consisting of:

between Gln157 and Lys158, between Glu172 and Asp173 and both of the aforementioned locations.

12. The affinity fluorescent protein expression cassette of Claim 11, wherein the recombinant peptide comprises the hexapeptide LEPRAS.

13. The affinity fluorescent protein of Claim 11 wherein the mutated GFP comprises a substitution of Ser147Pro.

14. An affinity fluorescent protein expression vector comprising a modified GFP nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein the modified GFP sequence comprises a heterologous amino acid sequence introduced at a position of the GFP molecule selected from the group consisting of: between Gln157 and Lys158, between Glu172 and Asp173 and both of the aforementioned locations.

15. The affinity fluorescent protein expression vector of Claim 14 wherein the mutated GFP comprises a substitution of Ser147Pro.

16. A host cell, comprising:
a recombinant nucleic acid molecule which comprises expression control sequences operatively linked to a nucleotide sequence encoding an affinity fluorescent protein, wherein said affinity fluorescent protein comprises a modified GFP molecule which comprises a mutated GFP molecule and a heterologous amino acid sequence which functions as a ligand-activated protein binding site, wherein the affinity fluorescent protein displays an altered spectral property when the binding site is engaged with ligand relative to the spectral property displayed when the binding site is not engaged by ligand.

17. The host cell of Claim 16 wherein the heterologous amino acid sequence is introduced at a location of the GFP molecule selected from the group consisting of: between Gln157 and Lys158, between Glu172 and Asp173 and both of the aforementioned locations.

18. The host cell of Claim 16 wherein the mutated GFP comprises a substitution of Ser147Pro.

19. A method of detecting the presence of a target ligand in a mixture of macromolecules comprising the steps of:

(a) preparing a sample to be evaluated for the presence of a target ligand molecule;

(b) contacting the sample of (a) with an affinity fluorescent protein which comprises a binding site for the target ligand;

(c) exciting the affinity fluorescent protein with light;

(d) measuring the fluorescent property that differs as a result of ligand activation of the affinity fluorescent protein.

20. The method of Claim 19, wherein the fluorescent property that differs as a result of ligand activation is selected from the group of properties consisting of: amplitude of the excitation, absorption or emission spectra and shape of the any of the aforementioned spectras.

21. The method of Claim 19 wherein the aFP comprises a modified fluorescent protein or molecule, such as a modified GFP molecule, which comprises a mutated GFP molecule and a heterologous amino acid sequence, thereby introducing a ligand-activated protein binding site, wherein the modified fluorescent protein displays an altered spectral property when the binding site is

engaged with ligand relative to the spectral property displayed when the binding site is not engaged by ligand.

22. The method of Claim 19 wherein the fluorescent property is measured using a solid support phase.

23. The method of Claim 22 wherein the solid support phase is selected from the group consisting of: nitrocellulose and a protein chip.

24. A method of detecting the occurrence of a target ligand in a cell comprising the steps of:

a) introducing into the cell an aFP which comprises a binding site for the target ligand;

(c) exciting the affinity fluorescent protein present in the cell with light;

(d) detecting a pattern of fluorescence due to ligand activation of the affinity fluorescent protein in the cell of (c) and comparing it to the pattern of fluorescence in a control cell,

wherein the pattern of fluorescence determines the occurrence of the target ligand in the cell.

25. The method of Claim 24 wherein the aFP comprises a modified fluorescent protein or molecule, such as a modified GFP molecule, which comprises a mutated GFP molecule and a heterologous amino acid sequence, thereby introducing a ligand-activated protein binding site, wherein the modified fluorescent protein displays an altered spectral property when the binding site is engaged with ligand relative to the spectral property displayed when the binding site is not engaged by ligand.

26. The method of Claim 24 wherein the cell is selected from the group consisting of: a macrophage and a yeast cell.

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